IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In Re: Patent Application

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| Applicant(s): | Maino et al. | | |
| Serial No.: | 08/803,702 | Group Art Unit: | 1644 |
| Filing Date: | February 21, 1997 | Examiner: | Gerald Ewoldt, Ph.D. |
| For: | METHOD FOR DETECTING T CELL RESPONSE TO SPECIFIC ANTIGENS IN WHOLE BLOOD | | |

APPEAL BRIEF UNDER 37 C.F.R. §41.37

Sir:

Appellants submit this brief in support of their notice of appeal dated July 30, 2004, pursuant to 37 C.F.R. §41.37.

Appellants file concurrently herewith the fee set forth in 37 C.F.R. §41.20(b)(2).

Appellants petition concurrently herewith under 37 C.F.R. §1.136(a) for a one month extension of time, extending time for filing the Brief to and including October 30, 2004.

In view of the arguments and authorities set forth below, this Board should find the Final Rejections of the appealed claims, as specified below, to be in error and should reverse them.

Table of Contents

**(i) Real party in interest**

The real party in interest consists of Becton, Dickinson and Company, which owns an undivided partial interest in the present application by virtue of an assignment from co-inventors Maino and Suni, and the Board of Regents, University of Texas System, which holds an undivided partial interest in the present application by virtue of an assignment from co-inventor Picker.

An assignment from co-inventors Maino and Suni to Becton, Dickinson and Company was recorded June 9, 1997 at Reel 8546, Frame 0073. An assignment from co-inventors Maino and Suni to Becton, Dickinson and Company also was recorded May 8, 2000 at Reel 010790, Frame 0936.

The assignment from co-inventor Picker to the Board of Regents, University of Texas System, was not recorded.

**(ii) Related appeals and interferences**

There are no prior or pending appeals, interferences or judicial proceedings known to Appellants, Appellants legal representative, or Assignee which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

**(iii) Status of claims**

The status of all the claims, pending or cancelled, is as follows:

Claims 19-24, 26-33, 35-55, and 61-66 are pending.

Claims 1-18, 25, 34, and 56-60 are canceled.

Claims 19-24, 26-33, 35-55, and 61-65 stand rejected.

Claim 66 is allowed.

The rejections of Claims 19-24, 26-33, 35-55, and 61-65 are being appealed.

**(iv) Status of amendments**

Appellant's amendment under 37 C.F.R. §41.33(b)(2) submitted on September 22, 2004 was entered. In response, an advisory action was issued October 14, 2002, stating that claim 66 now is allowed.

**(v) Summary of claimed subject matter**

Appellants' invention is in the field of immunology and provides methods for identifying and quantifying T lymphocytes (equivalently "T cells") that are specific for a particular antigen of interest, such as from an infectious agent (e.g., a virus) or administered in a vaccine. To aid in understanding the claimed invention, Appellants' first provide, as background, a summary of terms and concepts from the field of immunology relevant to the claimed invention. Appellants then provide a concise explanation of the subject matter defined in each of the independent claims involved in the appeal, which are claims 19, 64, and 65.

Background

T cells (equivalently "T lymphocytes") are groups of blood cells that are involved in the immune system's antigen-specific response to foreign (i.e., non-self) antigens. T cells are among the subclass of blood cells referred to as peripheral blood mononuclear cells (PBMC's).

Each T cell recognizes a specific antigen (its "cognate antigen") through a receptor on its surface, termed the "T cell receptor" ("TCR"), capable of binding to the antigen. Although T cells collectively recognize a phenomenal diversity of foreign antigens, each individual T cell recognizes a single discrete antigen, the specificity of recognition resulting from the specificity of the TCR-antigen binding. A T cell that recognizes a particular antigen is said to be specific for the antigen. The present invention relates to detecting those small number of T cells that recognize a specific antigen out of the totality of T cells present in a sample of blood (or PBMC-containing fraction of the blood).

T cell antigen binding is mediated by antigen presenting cells (APC's), also among the PBMC's, which process protein antigens into peptide fragments, complex the peptide fragments with major histocompatibility ("MHC") molecules, and display the resulting complexes on the cell surface. TCR's only bind peptide fragments of proteins that are complexed with MHC molecules and displayed by an APC. Antigens that are processed within an APC and displayed on the surface complexed with MHC molecules

are referred to as "nominal antigens" and are the antigens that evoke a classical antigen-specific immune response by T cells. The present invention, more particularly, relates to detecting those small number of T cells that recognize a specific nominal antigen out of the totality of T cells present in a sample of blood.

Binding of a T cell to its cognate antigen complexed with MHC molecules on the surface of an APC leads to activation of the T cell. Activation triggers biochemical and morphological changes in the T cell, including changes in protein expression, that culminate in differentiation of T cells into one of various types of effector cells.

Two classes of molecules whose expression are changed during activation are (1) cytokines, which are signaling proteins secreted from the T cell that mediate the immune response, and (2) proteins that appear on the surface of the activated T cell surface, referred to as activation antigens.[1] Detection of activation-induced protein expression facilitates identification of activated T cells. However, the two classes of molecules do not identify the same T cell population. In particular, CD69, an early activation antigen, is expressed within hours of T cell activation. However, only a subset of the CD69-expressing cells go on to secrete cytokines and fully mature into effector cells (see, e.g., the specification at page 8, lines 12-21). The methods of the present invention relate to the detection of the antigen-specific T cells that produce certain cytokines in response to stimulation with the nominal antigen.

In addition to presenting a nominal antigen bound to MHC molecules for recognition by a T cell, an APC also presents cell-surface molecules that contribute to the activation of the T cell. These cell surface molecules are said to provide costimulation. The addition of additional costimulatory molecules into the incubation buffer to further augment T cell activation is referred to exogenous costimulation.

T cells can be activated in other ways than with a nominal antigen presented by an APC. One class of activating agents, referred to polyclonal activators (equivalently, "mitogens"), bypass the T cell receptor and activate large fractions of T cells regardless of the T cells' antigen specificity. Another class of activating agents, termed

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[1] The nomenclature is somewhat confusing: an activation antigen, which is a protein expressed on the surface of an activated T cell, should not be confused with a nominal antigen, which is a protein recognized by a T cell in a process that leads to activation.

superantigens, activate large subsets of T cells without regard to the T cell's antigen specificity. Thus, the subset of T cells identified by detecting activation-induced protein expression depends on the class of agent that evoked activation. In the methods of the present invention, T cells specific for a nominal antigen are detected as those T cells that produce certain cytokines in response to stimulation with the nominal antigen.

The methods of the present invention are distinguished in part from prior art methods by both the class of agent used to evoke activation and the subset of T cells detected. The closest prior art of record describes methods for detecting T cells activated in a non-specific manner by detecting the expression of intracellular cytokines following stimulation by contact with a polyclonal activator or superantigen, which activates a vastly larger subset of the T cells in a sample. In these prior art methods, an inhibitor of cytokine secretion was used to allow intracellular cytokines to accumulate in the T cells, thereby facilitating the detection of the intracellular cytokines.

In the present invention, the frequencies of antigen-specific T cells are assessed flow cytometrically. Flow cytometry is a well known method of analyzing cells essentially one at a time that involves passing the cells in a fluid stream past a detection region and measuring various features of each cell as it passes. Prior to analysis, particular cellular components, such as intracellular or cell-surface proteins, are made visible to the detectors by labeling, typically with fluorophores (fluorescent dyes). The labeling of a particular protein in or on a cell is carried out using an antibody that binds specifically to this protein; typically the antibody is conjugated, directly or indirectly, to a fluorophore. Thus, the detection of a particular protein is achieved by adding a protein-specific antibody to the sample and detecting the binding of the antibody to the protein, which binding indicates the presence of the protein. Similarly, subsets of cells defined by the expression of a particular protein can be identified by adding an antibody specific to the subset-defining protein (also referred to as a subset-defining antibody) and detecting the binding of the antibody to the protein.

Using multiple antibodies specific to different proteins, each specificity labeled with a measurably distinct fluorophore, multiple proteins in a cell can be detected simultaneously. During a flow cytometric analysis, as a cell, which may have one or more of its proteins labeled, passes through the detection region, the cell is exposed to an

excitation light that causes any fluorophore labels, if present, to fluoresce (emit light), and the resulting fluorescent emission is measured as an indication of the presence of labeled protein. The amount of fluorescence, i.e., the intensity, observed from a fluorophore used to label a cellular protein corresponds to the amount of that protein in or on the cell. In addition, excitation light scattered by the cell itself typically is measured and provides additional information characteristic of the cell type.

Flow cytometric analysis yields data in the form of a set of emission intensity values measured for each cell, both from light scattered by the cell and from fluorescence emissions from the various labels. The detection of a cell by the detector also is referred to as an event, and measuring the cell parameters also is referred to as measuring events. Although a number of cellular parameters can be measured simultaneously, the resulting data typically are displayed two parameters at a time in a dot-plot in which each cell (event) is represented as a point with coordinates of the point corresponding to two intensity values. Examples of such dot-plots are provided in Figures 1-3 or the specification. Cell frequency within a subset is assessed by counting the number of cells of the desired type, each identified as having measured emission values characteristic of the subset, and comparing that the total number of cells observed.

Subject Matter of the Independent Claims

Of the pending claims, claims 19 and 64-66 are independent. Claim 66 is allowed and is not under appeal.

Claim 19

Independent claim 19 is drawn to a method of detecting T lymphocytes ("T cells") that are specific for a nominal antigen. The method of the present invention is described in broad terms in the specification at page 4, lines 10-22, and in originally filed claim 1. Claim 19 further recites additional steps, as described below. Claim 19, with steps numbered for reference, is shown below:

Claim 19. A method of detecting T lymphocytes that are specific for a nominal antigen, comprising:

1. culturing a sample containing peripheral blood mononuclear cells with a nominal antigen;
2. adding to said sample an inhibitor of cytokine secretion;
3. permeabilizing said cells;
4. adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and then
5. flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset.

In step 1 of claim 19, a sample containing peripheral blood mononuclear cells ("PBMC") and, thus, including both lymphocytes and antigen-presenting cells, is cultured with a nominal antigen. T cells that are specific for the stimulating nominal antigen are activated and begin to synthesize cytokines.

In step 2 of claim 19, an inhibitor of cytokine secretion is added, which results in the intracellular cytokines expressed within the activated T cells to accumulate, thereby enhancing detection. This step is described in the specification at page 5, lines 15-17.

In step 3 of claim 19, cells are permeabilized to allow antibodies added to the sample (in the following step) to enter the interior of the cell, thus enabling antibodies specific for intracellular proteins to bind to their target proteins, if present. This step is described in the specification as part of the preferred methodology at page 7, step 4.

In step 4 of claim 19, at least one cytokine-specific antibody and at least one T cell subset-defining antibody are added to the sample. This step is described as part of the general method at page 4, lines 10-15, and as part of the preferred methodology at page 7, step 6. T cell subset-defining antibodies, which bind to T cells independently of their activation status, may be chosen to bind, for example, to a protein expressed on all T cells, such as the CD3, or may, in addition or in the alternative, be chosen to bind to a protein is expressed on a smaller subset of T cells, such as CD4 or CD8, as described in the specification at page 3, lines 7-15; page 8, lines 15-18; and page 12, lines 10-20.

Examples of cytokine-specific antibodies are antibodies specific for IL 2, IL-4, IL-13, γ-IFN, or TNF-α, as disclosed in the specification at page 3, lines 7-15.

In step 5 of claim 19, flow cytometry is used to detect simultaneously the intracellular binding of the cytokine-specific antibody and the binding of the subset-defining antibody. Cells to which both types of antibodies bind are identified as T cells (or subset thereof) and as activated. The purpose of the method, the detection of T cells specific for the nominal antigen, is achieved because only T cells that are activated by the nominal antigen express the intracellular cytokines that are detected through the binding of the cytokine-specific antibodies. The detection step is described in the specification as part of the general method at page 4, lines 10-15, and as part of the preferred methodology at page 7, step 8, and in Example 3.

Claim 64

Claims 64 corresponds to claim 19 further limited to the use of the preferred inhibitor of cytokine secretion, Brefeldin-A, as described in the specification at page 5, lines 17, and exemplified throughout.

Claim 65

Claim 65 corresponds to claim 64 further narrowed to the method in which the step of culturing a sample containing peripheral blood mononuclear cells with a nominal antigen in the presence of Brefeldin-A is carried out in a slant tube, as in the preferred methodology described in the specification at page 6, step 3.

**(vi) Grounds of rejection to be reviewed on appeal**

Ground 1: Claims 19-24, 26-33, 35-36, 40-55, and 61-63 were rejected under 35 U.S.C. §112, first paragraph, for lack of adequate written description.

Ground II. Claims 19-24, 26-33, 35-55, and 61-65 were rejected under 35 U.S.C. §112, first paragraph, for lack of enablement.

**(vii) Argument.**

Ground I. The rejection of claims 19-24, 26-33, 35-36, 40-55, and 61-63 under the written description requirement of 35 U.S.C. §112, first paragraph.

Claims 19-24, 26-33, 35-36, 40-55, and 61-63 were rejected under the written description requirement of 35 U.S.C. §112, first paragraph, on the ground that the specification provides insufficient written description for the term "inhibitor of cytokine secretion." It is the examiner's position that this term potentially could encompass a large number of chemical compounds, and suggested that the claimed genus has not been adequately described.

The legal standard

Section 112 of the patent statute sets forth that "The specification shall contain a written description of the invention". 35 U.S.C. §112, first paragraph. This requirement is separate and distinct from the enablement requirement. *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991).

An objective standard for determining compliance with the written description requirement is, "does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989). Under *Vas-Cath Inc. v. Mahurkar*, supra, to satisfy the written description requirement, an applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, and that the invention, in that context, is whatever is now claimed.

It is well established that the description need only describe in detail that which is new or not conventional. *Hybritech v. Monoclonal Antibodies*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986). This is equally true whether the claimed invention is directed to a product or a process.

Where claims are drawn to novel combinations or uses of known compounds, in contrast to claims drawn to classes of new compounds per se or claims drawn to processes using those new compounds, the specification need not describe in detail the

known compounds themselves. *In re Fuetterer*, 319 F.2d 259, 138 USPQ 217, (CCPA 1963), discussed in further detail, below.

Claims 19-24, 26-33, 35-36, 40-55, and 61-63

Claims 19-24, 26-33, 35-36, 40-55, and 61-63 stand or fall together with respect to the present rejection. Claim 19 is in independent form; claims 20-24, 26-33, 35-36, 40-55, and 61-63 depend directly or indirectly from claim 19.

The specification provides literal written description of the invention using an inhibitor of cytokine secretion, and exemplifies the use of the preferred inhibitor, Brefeldin A ("BFA"). In particular, the invention using an inhibitor of cytokine secretion is described in the specification at page 5, lines 15-17:

> Further, it has been found intracellular cytokine detection is enhanced when an agent which blocks the secretion of such intracellular cytokines is added during the activation (4h) period of incubation.

In addition, written description of the method of the invention using an inhibitor of cytokine secretion is found in original claim 13, as filed.

> Original Claim 13: The method of Claim 1 further comprising adding a reagent which blocks secretion of intracellular cytokines during the culturing of the sample with the antigen specific stimulus.

The literal description of the invention as claimed in claim 19, using an inhibitor of cytokine secretion, provided both in the specification and the claims as filed conveys with reasonable clarity to those skilled in the art that, as of the filing date, the inventors were in possession of the invention as claimed in claim 19.

Despite the written description provided by the specification showing that the inventors were in possession of the claimed invention, the examiner rejected the claims for lack of written description of the class of compounds encompassed with "an inhibitor of cytokine secretion". Appellants' contend that the rejection is erroneous for the following reasons, discussed more fully below:

1. The use of an inhibitor of cytokine secretion to allow intracellular cytokines to accumulate, thereby facilitating the detection of the intracellular cytokines, was known at the time of the invention. The description need only describe in detail

that which is new or not conventional (*Hybritech v. Monoclonal Antibodies*, supra).

2. The claims, read as a whole, are drawn to a new use of known compounds (an inhibitor of cytokine secretion) and are *not* drawn to either novel compounds per se or to methods using novel compounds. In such a case, the applicant is not required to discover all the compounds from this class that would be useable in the methods (*In re Fuetterer*, supra).

The use of an "inhibitor of cytokine secretion" to facilitate detection of intracellular cytokines was known in the art

The invention of independent claim 19 is a method involving the detection of T cells activated in an antigen-specific manner by detecting the expression of intracellular cytokines following stimulation by contact with a nominal antigen. In the claimed method, an inhibitor of cytokine secretion is used to allow intracellular cytokines to accumulate, thereby facilitating the detection of the intracellular cytokines.

The closest prior art of record describes methods involving the detection of T cells activated in a non-specific manner by detecting the expression of intracellular cytokines following stimulation by contact with a polyclonal activator or superantigen. In these prior art methods, an inhibitor of cytokine secretion was used to allow intracellular cytokines to accumulate, thereby facilitating the detection of the intracellular cytokines. References of record that describe such methods, along with the particular inhibitor of cytokine secretion used, are provided in the table, below.

| **Inhibitor used** | **Reference** [2] |
|---|---|
| Monensin | Jung et al., 1993, J. Immunol. Methods 159:197-207 |
| Monensin | Elson et al., 1995, J. Immunol. 154(9):4294-4301 |
| Monensin | Prussin et al., 1995, J. Immunol. Methods 188:117-128 |
| Brefeldin-A | Picker et al., 1995, Blood 86:1408-1419 |
| Brefeldin-A & monensin | Application Note 1: Detection of Intracellular Cytokines in Activated Lymphocytes, Becton Dickinson and Co. |

[2] Submitted with the Information Disclosure Statement dated March 8, 1999, with the exception of Elson et al., submitted with the Supplementary Information Disclosure Statement dated December 1, 2000.

Each of the references listed in the table, above, describe methods involving the detection of intracellular cytokines in which an inhibitor of cytokine secretion is used to allow intracellular cytokines to accumulate in order to facilitate detection. Although the claimed methods are distinguished from these earlier methods both in the specificity of activation and the subset of T cells detected, the claimed methods include the use of an inhibitor of cytokine secretion for the same purpose as previously described, to allow intracellular cytokines to accumulate in T cells in order to facilitate detection. This particular element of the present invention is old in the art and conventional to one of skilled in the art of detecting intracellular cytokines[3].

The rejection is in error because it is based on an improper requirement to describe in great detail an element of the invention which is old in the art. Furthermore, the rejection conflicts with the precedent set by the Court in *Hybritech v. Monoclonal Antibodies*, supra, "The description need only describe in detail that which is new or not conventional". For at least this reason, Appellants submit that the rejection is erroneous and should be overturned.

The claims are drawn to a new use of known compounds

Independent claim 19 is drawn to a novel method that includes the known use of an inhibitor of cytokine secretion, not to the discovery of compounds useful as inhibitors of cytokine secretion. The examiner acknowledged that the present invention is a new use of old elements:

> In fact, none of the individual pieces of the claimed method are actually new [...] What is new is the *combination* of techniques and steps that achieve an unexpected result, i.e., the detection of cytokines that were thought to exist at levels below the threshold of detectability.

Last Office action, page 3, first paragraph. Thus, the claims are analogous to those at issue in *In re Fuetterer*, supra, wherein the Court clarified the written description requirement with respect to claims drawn to novel combinations or uses of known

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[3] Appellants further note that the fact that this element is old in the art is not in dispute. The examiner stated, "In fact, none of the individual pieces of the claimed method are actually new [...] What is new is the *combination* of techniques and steps that achieve an unexpected result, i.e., the detection of cytokines that were thought to exist at levels below the threshold of detectability." Last Office action, page 3, first paragraph.

compounds, in contrast to claims drawn to classes of new compounds per se or claims drawn to processes using those new compounds.

In *In re Fuetterer*, claims drawn to a rubber stock composition useful in producing tire treads included a recitation of "an inorganic salt capable" of maintaining an homogeneous distribution of another component in the composition. The disclosure listed the function desired and four members of the class having that function. The claims had been rejected by the examiner as being overly broad ("'inorganic salt" reads on literally thousands of materials, many of which would not be operative for applicant's purpose'. Ibid at 220). The board agreed, noting that rejection was based on "the inordinate breadth of the claimed salts when it is not apparent from the disclosure of only four salts what other salts would be suitable to serve the function asserted and required by the claims" (Ibid at 220, 221). However, the Court overturned the rejection and found the written description requirement to be satisfied:

> Appellant's invention is the combination claimed and not the discovery that certain inorganic salts have colloid suspending properties. We see nothing in patent law which requires appellant to discover which of all those salts have such properties and which will function properly in his combination. The invention description clearly indicates that any inorganic salt which has such properties is usable in his combination. If others in the future discover what inorganic salts additional to those enumerated do have such properties, it is clear appellant will have no control over them per se, and equally clear his claims should not be so restricted that they can be avoided merely by using some inorganic salt not named by appellant in his disclosure.

Ibid at 223 (USPQ pagination) (emphasis added). Appellants submit that the facts in the present case are analogous to those in *In re Fuetterer*.

As in *In re Fuetterer*, the present claims stand rejected in view of the large number of potential inhibitors of cytokine secretion. Analogously, the present claims are to a combination of steps, one being the known use of an inhibitor of cytokine secretion, not to the discovery of compounds that act as inhibitors of cytokine secretion. In accord with in *In re Fuetterer*, nothing in patent law requires Appellants to discover which of all those potential inhibitors of cytokine secretion have such properties and which will function properly in the claimed combination of steps. Furthermore, if others in the future discover another suitable inhibitor of cytokine secretion, the present claims should not be so restricted that they can be avoided merely by using some inhibitor of cytokine

secretion not described in the specification. The rejection is in error because it is based on an erroneous standard and is at odds with the precedent set by the Court in *In re Fuetterer*. For at least this reason, Appellants submit that the rejection is erroneous and should be overturned.

Appellants suggest an analogous fact pattern that may clarify the error of the rejection. Consider hypothetical claims drawn to a novel multi-step method of treating a patient in which one step is treating a headache using a pain reliever, supported in the specification by a teaching to use "a pain reliever, such as aspirin." The use of a pain reliever to treat a headache is, of course, well known and several suitable compounds are commercially available; the teaching of the specification would be sufficient to convey to one of skill that this step can be carried out using any of suitable pain relievers known. While it is true that only a very small number of medically suitable compounds are known out of the "infinity" of potential compounds, and, in fact, the full scope of the term "pain reliever" is not known (hence, the continuing research into new compounds), the hypothetical invention simply comprises the use of a known pain-reliever for its known purpose. The teaching in the present specification to use an inhibitor of cytokine secretion, such as BFA, is analogous to a teaching in the hypothetical specification to use a pain reliever, such as aspirin - both teach the use of a known compound to carry out a known step. The present rejection is analogous to an improper requirement that the inventor of this hypothetical invention describe in detail the class of compounds suitable for use as a pain reliever.

In summary, the literal description in the specification reasonably conveys to one of skill in the art that Appellants were in possession of methods using "an inhibitor of cytokine secretion". This element of the claimed invention, specifically the use of an inhibitor of cytokine secretion to achieve an accumulation of intracellular cytokines to facilitate detection, was known at the time of the invention; the specification need not describe this known element in detail. Furthermore, the claims are to a combination of elements, one being the known use of an inhibitor of cytokine secretion, not to the discovery of compounds that act as inhibitors of cytokine secretion; again, the

specification need not describe this known element in detail. The rejection is based on an erroneous standard that conflicts with the precedent of the case law cited.

For the reasons discussed herein and in view of the case law cited, Appellants submit that the specification fully meets the written description requirement. Appellants respectfully request that the Board overturn the rejection of claims 19-24, 26-33, 35-36, 40-55, and 61-63 under the written description requirement of 35 U.S.C. §112, first paragraph.

Ground II. The rejection of claims 19-24, 26-33, 35-55, and 61-65 under the enablement requirement of 35 U.S.C. §112, first paragraph.

Claims 19-24, 26-33, 35-55, and 61-65 were rejected under 35 U.S.C. §112, first paragraph, for lack of enablement, based on the grounds that a disclosed critical limitation is missing from the claims. As stated in the Office action dated April 28, 2004 (referred to herein as "the last Office action"):

> Elements critical or essential to the practice of the invention, but not included in the claims are not enabled by the disclosure, for the reasons of record.

Last Office action, page 4, §5.

However, which elements are alleged to be critical is unclear. In restating the "reasons of record", the examiner restated the rejection as set forth in the Office action dated December 2, 2002, and additionally provided comments responding specifically to the arguments set forth in Appellants' amendment dated February 17, 2004. No acknowledgement was made of the prosecution history in the interim, namely Appellants' amendments dated March 31, 2003 and September 3, 2003, and the Office actions dated June 13, 2003 and October 14, 2003. It is Appellants' understanding that the examiner had agreed that particular features alleged to be critical in the Office action dated December 2, 2002 are, in fact, not critical features and, thus, Appellants' believe their mention in the restated reasons for the rejection was unintentional.

Clarity as to the particular elements alleged to be critical is necessary in order for Appellants to respond to the rejection with appropriately grouped claims, as the elements are incorporated into the claims in various combinations. Thus, both for completeness and in order to be able to group claims, Appellants first address all elements mentioned in the restatement of the rejection and summarize Appellants' understanding of the reasons for the rejection.

Appellants' understanding of the reasons for the rejection

The reasons for the rejection restated from the Office action dated December 2, 2002 (last Office action, §5, paragraph spanning pages 4-5) included the following elements as allegedly critical elements omitted from one or more claims:

1. the step of culturing the sample with the antigen;

2. added costimulation ("as recited in Claim 20");

3. the inclusion of an inhibitor of cytokine secretion; and

additional required steps described in Example 4, "for example"

4. the use of slant tubes

5. the inclusion of CD69 (not just any activation marker)

6. the collection of at least 50,000 events

Of these elements, Appellants believe that elements 1, 2, and 5 are not presently alleged to be critical, for the reasons summarized below.

Regarding element 1, the step of culturing the sample with the antigen, claim 19 was amended to recite the step of culturing the sample with the nominal antigen in the amendment dated March 31, 2003. The examiner acknowledged the amendment and explicitly withdrew this as a reason for the rejection in the Office action dated June 13, 2003 (page 4, last paragraph). Appellants understand that this element is not alleged to be an omitted critical element.

Regarding element 2, the step of adding (i.e., exogenous) costimulation, the examiner earlier had stated, without support, that "it is well-established that antigen stimulation in the absence of costimulation (....) will result in anergy (not activation)" (Office action dated December 2, 2002, page 5). In the amendment dated March 31, 2003, Appellants pointed out that costimulation inherently is provided by antigen presenting cells (APC) in the sample, making the addition of an exogenous costimulant (as recited in Claim 20) optional. This element was not cited as a reason for the rejection in the subsequent Office actions dated June 13, 2003 and October 14, 2003, and Appellants understand that this element is no longer alleged to be critical.

Regarding the additional steps described in Example 4, this example describes four key areas in which the methods of the invention, which involve detecting T cell cytokine expression in response to a nominal antigen, differ from the prior art methods that involve detecting T cell cytokine expression in response to polyclonal stimulators: (1) the geometry of the T cell/accessory cell interaction (specification at page 16, lines 13-16); (2) the timing of the addition of Brefeldin-A and the use of exogenous costimulation (specification at page 16, lines 16-18); (3) assessment of CD69 (specification at page 16, lines 18-26); and (4) the number of events collected

(specification at page 16, line 26 to page 17, line 2).[4] Appellants provided arguments showing that the teaching of Example 4, and, in particular, regarding these 4 areas, is not a description of critical elements whose omission would result in the methods being wholly inoperable (restated in the amendment dated February 17, 2004). In responding to Appellants arguments, the examiner explicitly stated that the timing of BFA inclusion and the assessment of CD69 (element 5, above) are not critical features because the specification does not disclose it a such (last Office action, page 6). Appellants understand that the use of exogenous costimulation also is not alleged to be critical, both for the reasons stated above and further because this feature is described in the same sentence in Example 4 together with the timing of BFA inclusion, which description the examiner stated does disclose a critical element.

Thus, it is Appellants understanding that the sole reasons for the rejection are the alleged criticality of

- the use of slant tubes
- the measurement of at least 50,000 events
- inclusion of an inhibitor of cytokine secretion

and that the alleged criticality is based on the description of these features in Example 4.

The legal standard

35 U.S.C. §112, first paragraph, sets forth that the specification, not the claims, must provide an enabling written description of the invention:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

35 U.S.C. §112, first paragraph.

The examiner cited *In re Mayhew*, 527 F.2d 1229, 1233, 188 USPQ 356, 358 (CCPA 1976) as supporting a rejection of claim under the enablement provision section of 35 U.S.C. §112 when a feature which is taught as critical in a specification and is not

[4] The last paragraph of Example 4 (page 17, lines 4-17) provides further guidance directed to a particular narrower embodiment of the invention in which the sample is whole blood. Appellants believe that this is not relevant to the rejection.

recited in the claims. In *In re Mayhew*, the specification contained multiple teachings that the invention was dependent on a specific feature (a cooling zone, specifically located), and this feature was omitted entirely in the broadest claim. The teachings of the specification, considered in its entirety, indicated that the best mode (i.e., with a cooling zone present) was, in fact, the only mode supported.

The applicability of the reasoning of *In re Mayhew* depends on a proper determination of whether a feature actually is a critical feature, i.e., whether the specification teaches that the invention truly depends on the inclusion of the critical feature. A determination that a disclosed limitation is critical should be made only when the language of the specification, taken as a whole, makes it clear that the limitation is critical for the invention to function as intended:

> In determining whether an unclaimed feature is critical, the entire disclosure must be considered. Broad language in the disclosure (including the abstract) omitting an allegedly critical feature tends to rebut the argument of criticality. Also, features that are merely preferred are not critical.

*In re Goffe*, 542 F.2d 564, 567, 191 USPQ 429, 431 (CCPA 1976). (citations removed, emphasis added). More recently, the Court further clarified that *In re Mayhew* dealt with a case in which an element was critical in the sense that its omission would result in the invention being wholly inoperative:

> [The dissent] cites *In re Mayhew* for the proposition that "claims failing to recite a necessary element of the invention fail for lack of an enabling disclosure." There, however, the method claims omitted a step without which the invention as claimed was wholly inoperative (meaning it simply would not work and could not produce the claimed product).

*Amgen Inc. v Hoechst Marion Roussel Inc.* 314 F.3d 1313, 65 USPQ2d 1385, 1403 (CAFC 2003) (emphasis added). Restated equivalently, a rejection as in *In re Mayhew* is properly applied only where the specification as a whole teaches than any embodiment omitting the critical element would be wholly inoperative, i.e., not enabled, and, in particular, is improperly applied if the specification teaches that the omitted feature is merely preferred.

An example of an improper rejection based on an improper determination of a feature as critical is found in *In re Johnson and Farnham*, 558 F.2d 1008, 194 USPQ 187 (CCPA 1977). Therein, the Court overturned a rejection based on the grounds that a

disclosed critical limitation was missing from the claims. The claims at issue were drawn to a chemical polymer. The specification taught that the invention required a minimum "sigma value" for one of the constituent subunits, but the minimum value was not recited in the claims. The examiner had rejected the claims for failing to recite the minimum value on the basis that this minimum value was a critical element of the invention, and the rejection had been upheld by the Board. However, the Court overturned the rejection, stating:

> The PTO would limit appellants to claims reciting a sigma value of at least 0.7. This view is improper because it requires the claims to set forth the practical limits of operation for the invention and it effectively ignores the scope of enablement provided by the specification as a whole.

*In re Johnson and Farnham* at 195 (USPQ pagination). The Court stated further, "it is the function of the specification, not the claims, to set forth the "practical limits of operation" of an invention" and, further, "[o]ne does not look to claims to find out how to practice the invention they define, but to the specification.". Ibid at 195 (citations omitted).

In summary, a rejection based omission of a critical element is improper unless the specification, taken as a whole, teaches that omission of the element would result in the invention being wholly inoperable. Elements that represent preferred embodiments, e.g., elements that optimize, maximize, or increase accuracy of the results, or elements that set for the practical limits of operation are not critical.

Claims 19-24, 26-33, 35-38, 40-55, and 61-63

Claims 19-24, 26-33, 35-38, 40-55, and 61-63 stand or fall together with respect to the present rejection. Claim 19 is in independent form; claims 20-24, 26-33, 35-38, 40-55, and 61-63 depend directly or indirectly from claim 19. Below, Appellants address each element that represents a reason for the rejection, as summarized, above, and show that the rejection is in error.

Inhibitor of cytokine secretion

The examiner stated that the specification and the post-filing art disclose/teach that the inclusion of an inhibitor of cytokine secretion is essential (last Office action, §5,

page 5). Claim 19 and, thus, claims dependent therefrom recite the inclusion of an inhibitor of cytokine secretion. The use of an inhibitor of cytokine secretion is not an omitted element. Thus, Appellants believe this element does not represent a reason for the rejection.

For completeness, Appellants point out that the specification fully enables the claimed method using an inhibitor of cytokine secretion. As discussed above under Ground I, the use of an inhibitor of cytokine secretion to allow the accumulation of cytokines within activated T cells was known in the art. One of skill in the art, following the teaching of the specification and without undue experimentation, could carry out the claimed methods using a known inhibitor of cytokine secretion.

The use of slant tubes

The rejection was based on the teachings of Example 4, which the examiner alleges teaches that the use of slant tubes is a critical element of the invention. This rejection is in error for any and all of the following reasons.

The specification describes the invention in broad terms omitting the use of slant tubes. For example, the invention is described in the specification omitting the use of slant tubes at page 4, lines 11-15, and in claim 1 as filed. This broad language in the disclosure omitting the allegedly critical features tends to rebut the argument of criticality.

Furthermore, the specification also clearly indicates that the teachings of the examples, including Example 4, are no more than preferred embodiments of the invention. In particular, the specification states at page 13, lines 16-17 with reference to all the examples, "The following examples illustrate certain preferred embodiments of the invention but are not intended to be illustrative of all embodiments" (emphasis added); and at page 18, lines 13-14, "The specific embodiments are given by way of example only,..."). This language in the specification further rebuts the argument of criticality by making clear that the teaching of Example 4 is of preferred embodiments. An element that is merely a preferred embodiment is not a critical feature without which the invention would be wholly inoperative.

Example 4, in distinguishing T cell responses to antigen (Ag) from T cell responses to mitogen and superantigen, states that "the geometry of the T cell/accessory cell interaction was critical for Ag responses; maximal responses were observed in slant tubes...." (page 16, lines 13-14, emphasis added). Appellants point out that the use of slant tubes, which provides one particular geometry of the T cell/accessory cell interaction, is described as maximizing the responses, not as a critical element whose omission would result in the invention being wholly inoperative. An element that merely maximizes responses represents a preferred embodiment and is not a critical feature without which the invention would be wholly inoperative.

For the reasons provided above, Appellants submit that the specification as a whole does not teach that the use of slant tubes is a critical element without which the invention would be wholly inoperative. In fact, the specification clearly describes the use of slant tubes as a preferred embodiment of the invention. An element that is merely a preferred embodiment is not a critical feature without which the invention would be wholly inoperative.

Finally, factual evidence of record demonstrates that the invention is operable, i.e., enabled, without the use of slant tubes. Suni et al., 1998, J. Immunol. Methods. 221:89-98[5] is Appellants' first publication in a peer-reviewed scientific journal of the methods of the present invention using whole blood samples. Suni et al. include a description of results obtained using the methods of the present invention carried out using culture tubes incubated upright (see §2.2), which demonstrates that the enablement of the invention is not limited to use of the slant tubes. Thus, Suni et al. provide data that demonstrates that the use of slant tubes is not a critical element without which the invention would be wholly inoperative.

The examiner improperly ignored the factual evidence of record on the basis that it was generated post-filing. It is well established that an applicant may provide post-filing data (for example, in a declaration under 37 C.F.R. §1.32) that demonstrates that the written description provided by the specification at the time of filing is, in fact, enabling. Appellants did not cite Suni et al. as augmenting the specification, rather

Appellants cited Suni et al. as describing data obtained using the methods of the invention as described in the "summary of the invention" of the specification as filed, which description does not included the use of slant tubes. Evidence of record that demonstrates that written description provided by the specification at the time of filing is, in fact, enabling must be considered. The examiner erred in ignoring this evidence that demonstrates that the claimed invention, carried out without the use of slant tubes, is enabled and, thus, refutes the allegation that use of slant tubes is a critical element without which the invention would be wholly inoperative. For this additional reason, the allegation that the collection of at least 50,000 events is a critical element of the invention is in error, and the rejection based on this determination is in error.

The number of events collected

The rejection was based on the teachings of Example 4, which the examiner alleges teaches that the use of collection of at least 50,000 events is a critical element of the invention. This rejection is in error for any and all of the following reasons.

The specification describes the invention in broad terms omitting the collection of 50,000 events. For example, the invention is described in the specification omitting the collection of 50,000 events at page 4, lines 11-15, and in claim 1 as filed. This broad language in the disclosure omitting the allegedly critical features tends to rebut the argument of criticality.

Furthermore, the specification also clearly indicates that the teachings of the examples, including Example 4, are no more than preferred embodiments of the invention. In particular, the specification states at page 13, lines 16-17 with reference to all the examples, "The following examples illustrate certain preferred embodiments of the invention but are not intended to be illustrative of all embodiments" (emphasis added); and at page 18, lines 13-14, "The specific embodiments are given by way of example only,..."). This language in the specification further rebuts the argument of criticality by making clear that the teaching of Example 4 is of preferred embodiments. An element

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[5] Submitted with the Information Disclosure Statement dated March 5, 1999. Appellants also provided Examiner with a copy of Suni et al., 1998, J. Immunol. Methods. 221:89-98 during the interview on August 20, 2003.

that is merely a preferred embodiment is not a critical feature without which the invention would be wholly inoperative.

Example 4 describes that "because of the relatively small size of the Ag-specific populations, accurate assessment of the responses required the routine collection and analysis of at least 50,000 events per determination (page 17, lines 1-2, emphasis added). Accuracy in the assessment of antigen-specific T-cell frequencies is the statistical problem of distinguishing a small subpopulation of positive events (antigen-specific T cells) from the "noise" of detecting a vastly larger population of negative events. As is well known, the accuracy of a statistical test typically is improved by the collection of a larger data set. Appellants submit that this teaching, which relates to the accuracy of the method, not the basic operability, represents guidance as to the practical limits of operation. It is a function of the specification, not the claims, to set forth the "practical limits of operation" of an invention.

Appellants further submit that an element that affects the accuracy of a method cannot be considered a critical element for claims in which a specific level of accuracy is not a claim element, as is the case here. The claims are drawn to methods of detecting antigen-specific T cells, not to methods having a prerequisite accuracy. Although accuracy may be important for a particular application, such as a commercial application, the level of accuracy is not an element of the claimed invention. Again, teaching that relates to the accuracy of the invention represents guidance as to the practical limits of operation, and it is the function of the specification, not the claims, to set forth the "practical limits of operation" of an invention.

Appellants further point out that Example 3 of the specification describes analyses were carried out using only 48,000 events (page 15, lines 15-16). The description in the specification of the invention using less than 50,000 events clearly demonstrates that the collection of 50,000 events is a not an element whose omission would result in the method being wholly inoperable. Thus, the specification itself clearly refutes the allegation that the collection of at least 50,000 events is a critical element of the invention.

The examiner improperly ignored Example 3 on the basis that it discloses no data (last Office action, page 6, penultimate paragraph). However, Example 3 is not a

prospective example; it clearly describes (in past tense) that actual experiments were carried out. It is an error for the examiner to selectively disregard the description in the specification of actual experiments simply because the data were not included - the inclusion of experimental data in the specification is not a requirement under U.S. patent law. Furthermore, it is an error for the examiner to selectively disregard sections of the specification, as the whole specification must be considered.

For the reasons provided above, Appellants submit that the specification as a whole does not teach that the collection of at least 50,000 events is a critical element without which the invention would be wholly inoperative. In fact, Example 3 clearly refutes the allegation that the collection of at least 50,000 events is a critical element of the invention. Furthermore, the specification clearly describes Example 4 as describing a preferred mode of the invention. An element that merely a preferred embodiment is not a critical feature without which the invention would be wholly inoperative. Furthermore, teaching relating to the accuracy of the invention represents guidance as to the practical limits of operation, and it is the function of the specification, not the claims, to set forth the "practical limits of operation" of an invention. For at least these reasons, the allegation that the collection of at least 50,000 events is a critical element of the invention is in error, and the rejection based on this determination is in error.

Finally, factual evidence of record demonstrates that the invention is operable, i.e., enabled, with fewer events collected. Suni et al. (cited above) describe results obtained using the methods of the invention in which 40,00-50,000 events were collected, which demonstrates that the enablement of the invention is not limited to the collection of at least 50,000 events (see Suni et al., §2.4). Thus, Suni et al. provide data that demonstrates that the collection of at least 50,000 events is not a critical element without which the invention would be wholly inoperative.

Again, the examiner improperly ignored the factual evidence of record on the basis that it was generated post-filing. It is well established that an applicant may provide post-filing data (for example, in a declaration under 37 C.F.R. §1.32) that demonstrates that the written description provided by the specification at the time of filing is, in fact, enabling. Appellants did not cite Suni et al. as augmenting the specification, rather Appellants cited Suni et al. as describing data obtained using the methods of the

invention as described in the "summary of the invention" of the specification as filed, which description does not included the collection of at least 50,000 events. Evidence of record that demonstrates that written description provided by the specification at the time of filing is, in fact, enabling must be considered. The examiner erred in ignoring this evidence that demonstrates that the claimed invention, carried out without the collection of at least 50,000 events, is enabled and, thus, refutes the allegation that use of slant tubes is a critical element without which the invention would be wholly inoperative. For this additional reason, the allegation that the collection of at least 50,000 events is a critical element of the invention is in error, and the rejection based on this determination is in error.

For the reasons discussed herein, and in view of the case law cited, Appellants maintain that claims 19-24, 26-33, 35-38, 40-55, and 61-63 are fully enabled by the specification and that the rejection is in error. Appellants respectfully request that the Board overturn the erroneous rejection of claims 19-24, 26-33, 35-38, 40-55, and 61-63 under the enablement requirement of 35 U.S.C. §112, first paragraph.

Claim 64

Claims 39 and 64 stand separately with respect to the rejection because only a subset of the reasons for the rejection could apply. Claim 39, which depends from independent claim 19, further recites the use of Brefeldin-A, the preferred inhibitor of cytokine secretion exemplified in the specification. Claim 64 corresponds to claim 39 presented in independent form. Thus, the only elements alleged by the examiner to be critical that are not recited in claims 39 and 64 are the use of slant tubes and the collection of at least 50,000 events.

As discussed above, the determination that the use of slant tubes and the collection of at least 50,000 events are critical features of the invention is in error and ignores the teaching of the specification as a whole. whose omission would result in the invention being wholly inoperable. The specification as a whole describes the element as a preferred embodiment. Furthermore, factual evidence of record (Suni et al., supra) provides evidence that the written description in the specification of the invention

omitting both the use of slant tubes and the collection of at least 50,000 events, is, in fact, enabling.

For the reasons discussed herein, and in view of the case law cited, Appellants maintain that claims 39 and 64 are fully enabled by the specification and that the rejection is in error. Appellants respectfully request that the Board overturn the erroneous rejection of claims 39 and 64 under the enablement requirement of 35 U.S.C. §112, first paragraph.

Claim 65

Claim 65 stands separately with respect to the rejection because only a subset of the reasons for the rejection could apply. Claim 65, in addition to reciting all elements of independent claim 19, further recites the use of Brefeldin-A, the preferred inhibitor of cytokine secretion exemplified in the specification, and the use of slant tubes. Thus, the one element alleged by the examiner to be critical that is not recited in claim 65 is the collection of at least 50,000 events.

As discussed above, the determination that the collection of at least 50,000 events are critical features of the invention is in error and ignores the teaching of the specification as a whole. Furthermore, factual evidence of record (Suni et al., supra) provides evidence that the written description in the specification of the invention omitting the collection of at least 50,000 events, is, in fact, enabling.

For the reasons discussed herein, and in view of the case law cited, Appellants maintain that claim 65 is fully enabled by the specification and that the rejection is in error. Appellants respectfully request that the Board overturn the erroneous rejection of claims 39 and 64 under the enablement requirement of 35 U.S.C. §112, first paragraph.

**(viii) Claims appendix**

1-18. (canceled)

19. A method of detecting T lymphocytes that are specific for a nominal antigen, comprising:

culturing a sample containing peripheral blood mononuclear cells with a nominal antigen;

adding to said sample an inhibitor of cytokine secretion;

permeabilizing said cells;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and then

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset.

20. The method of claim 19, further comprising the step of adding to said sample, contemporaneously with antigen contact, a costimulus of T cell activation.

21. The method of claim 20, wherein said costimulus is an antibody specific for CD28.

22. The method of claim 20, wherein said costimulus is an antibody specific for VLA-4.

23. The method of claim 19, further comprising contacting said sample with an antibody specific for a T lymphocyte early activation antigen, and then flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset that concurrently bind said early activation antigen-specific antibody.

24. The method of claim 23, wherein said T lymphocyte early activation antigen is CD69.

25. (canceled)

26. The method of any one of claims 19, 20, 23, or 25 wherein said sample is a whole blood sample.

27. The method of claim 26, further comprising the step of adding a cationic chelator after antigen contact is complete but prior to flow cytometric detection.

28. The method of claim 27, wherein said chelator is EDTA or EGTA.

29. The method of claim 28, wherein said chelator is EDTA.

30. The method of claim 26, further comprising the step of lysing red blood cells.

31. The method of claim 19, wherein said nominal antigen is selected from the group consisting of alloantigens, autoantigens, viral antigens, and bacterial antigens.

32. The method of claim 31, wherein said nominal antigen is a viral antigen.

33. The method of claim 32, wherein said antigen is a CMV antigen.

34. (canceled)

35. The method of claim 32, wherein said antigen is a mumps antigen.

36. The method of claim 32, wherein said antigen is a measles antigen.

37. The method of claim 31, wherein said MHC-dependent nominal antigen is a bacterial antigen.

38. The method of claim 37, wherein said antigen is a Mycobacterium tuberculosis antigen.

39. The method of claim 19, wherein said inhibitor of cytokine secretion is Brefeldin A.

40. The method of claim 19, wherein said cytokine-specific antibody is specific for a cytokine selected from the group consisting of: IL-2, IL-4, IL-13, γ-IFN, and TNF-α.

41. The method of claim 40, wherein said cytokine-specific antibody is specific for IL-2.

42. The method of claim 40, wherein said cytokine-specific antibody is specific for IL-4.

43. The method of claim 40, wherein said cytokine- specific antibody is specific for γ-IFN.

44. The method of claim 40, wherein said cytokine-specific antibody is specific for TNF-α.

45. The method of claim 19, wherein said T lymphocyte subset-defining antibody is selected from the group consisting of antibodies specific for: CD3, CD4, CD8, TCR, homing receptors, CD45RO, CD45RA and CD27.

46. The method of claim 45, wherein said T lymphocyte subset-defining antibody is specific for CD3.

47. The method of claim 45, wherein said T lymphocyte subset-defining antibody is specific for CD4.

48. The method of claim 45, wherein said T lymphocyte subset-defining antibody is specific for CD8.

49. The method of any one of claims 19, 20, or 23 wherein said anti-cytokine antibodies, said T lymphocyte subset-defining antibodies, and said early activation antigen-specific antibodies are each conjugated directly to fluorophores.

50. The method of claim 49, wherein said fluorophores are selected from the group consisting of FITC, PE, PerCP, and APC.

51. The method of claim 50, wherein said anti-cytokine antibodies are conjugated to FITC.

52. The method of claim 50, wherein said T lymphocyte subset-defining antibodies are conjugated to PerCP.

53. The method of claim 50, wherein said antibody specific for a T lymphocyte early activation antigen is conjugated to PE.

54. The method of any one of claims 19, 20, or 23 wherein said antigen-contacting step lasts no longer than 24 hours.

55. The method of claim 54, wherein said antigen-contacting step lasts no longer than 6 hours.

56-60. (canceled)

61. The method of claim 19, wherein each of said at least one cytokine-specific antibody is specific for a cytokine selected from the group consisting of IL-2, IL-4, IL-13, IFN-γ, and TNF-α.

62. The method of claim 61, further comprising the step of adding to said sample, contemporaneously with antigen contact, a costimulus of T cell activation, wherein said costimulus is selected from the group consisting of antibodies specific for CD28, VLA-4, CD86, or CD118.

63. The method of claim 61, further comprising contacting said sample with an antibody specific for CD69, and then flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by CD69+ cells in the defined T lymphocyte subset.

64. A method of detecting T lymphocytes that are specific for a nominal antigen, comprising:

culturing a sample containing peripheral blood mononuclear cells with a nominal antigen in the presence of Brefeldin-A;

permeabilizing said cells;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset.

65. A method of detecting T lymphocytes that are specific for a nominal antigen, comprising:

culturing a sample containing peripheral blood mononuclear cells with a nominal antigen in the presence of Brefeldin-A, wherein said culturing is carried out in a slant tube;

permeabilizing said cells;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset.

66. (**allowed - not involved in the appeal**) A method of detecting T lymphocytes that are specific for a nominal antigen, comprising:

culturing a sample containing peripheral blood mononuclear cells with a nominal antigen in the presence of Brefeldin-A, wherein said culturing is carried out in a slant tube;

permeabilizing said cells;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset, wherein said step of flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset comprises analyzing at least 50,000 cells.

**(ix) Evidence appendix**

None of the evidence submitted pursuant to 37 C.F.R. §§1.130, 1.131, or 1.132 during the prosecution of the present application are being relied upon for the arguments presented herein.

**(x) Related proceedings appendix**

There are no related proceedings identified pursuant to 37 C.F.R. §41.37(c)(1)(ii).

**(xi) Table of Authorities**

## Cases